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<110> Korea Research Institute of Bioscience and Biotechnology

<120> Method for screening of a lipase having improved enzymatic activity using yeast surface display vector and the lipase

<130> 3fpo-07-05

<150> KR 2002-55575

<151> 2002-09-13

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<213> Artificial Sequence

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27

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<220>

<223> CALB primer 2

<400> 2

gcggatcctc agggggtgac gat

23

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<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> CALB primer 3

<400> 3

gcggatccgg gggtagcgat gccggag

27

<210> 4

<211> 19

<212> DNA

<213> Artificial Sequence

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<223> GPD-err primer

<400> 4

gcagagctaa ccaataagg

19

<210> 5

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> T-0 primer

<400> 5

tgcaattgaa cacaaccac

19

<210> 6

<211> 1023

<212> DNA

<213> Candida antarctica

<220>

<221> sig_peptide

<222> (-51)..(-1)

<223> secretion signal

<400> 6

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ttgtgaagc gtctgccttc cgggtcggac cctgcctttt cgcagcccaa gtcggtgctc 69

gatgcgggtc tgacctgcca ggggtccttc ccatcctcgg tctccaaacc catcctctc 129

gtccccgga cgggcaccac aggtccacag tcgttcgact cgaactggat cccctctct 189

gcgcagctgg gtacacacc ctgctggatc tcacccccgc cgttcattgt caacgacacc 249

caggtaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 309

aacaagcttc ccgtgctcac ctgtcccag ggtggtctgg ttgcacagtg gggctgacc 369

ttctcccca gtatcaggtc caaggtgat cgacttatgg cctttcgcc cgactacaag 429

ggcaccgtcc tcgcggcccc tctcgatgca ctgcgggta gtgcaccctc cgtatggcag 489

caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtgggtct gacccagatc 549

gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac 609

tcgccactcg actcatccta cctcttcaac gggaagaacg tccaggcaca ggctgtgtgt 669

gggccgctgt tcgtcatcga ccatgcaggc tcgtcacct cgcagttctc ctacgtcgtc 729

ggtcgatccg ccctgcgctc caccacgggc caggctcgta gtgcagacta tggcattacc 789

gactgcaacc ctctcccg ccaatgatctg actcccgagc aaaaggctgc cgcggctgcg 849

ctccggcgcg cggcggctgc agccatcgtg gcgggtccaa agcagaactg cgagcccgac 909

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ccc 972

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<212> DNA

<213> Candida antarctica

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<221> sig_peptide

<222> (-51)..(-1)

<223> secretion signal

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gatgcgggtc tgacctgcca aggtgcttcg ccatcctcgg totccaaacc catccttctc 129

gtccccgga cggcaccac aggtccacag tegtctgact cgaactggat cccctctct 189

gcgcagctgg gttacacacc ctgctggatc tcacccccgc cgtcatgct caacgacacc 249

caggtaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttggggcaac 309

aacaagctc ccgtgtcac ctggtccag ggtggtctgg ttgcacagt ggtctgacc 369

ttctccca gtatcaggtc caaggctgat cgactatgg ccttgcgc cgactacaag 429

ggcaccgtcc tcgccggccc tctgatgca ctgcggta gtgcaccctc cgtatggcag 489

caaaccaccg gttcggcact cactaccgca ctccgaaacg cagggtgtct gaccagatc 549

gtgccacca ccaacctta ctgcggacc gacgagatcg ttcagcctca ggtgtcaac 609

tgcacctcg actcatccta cctttcaac ggaagaacg tcaggcaca ggctgtgtgt 669

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ggtgatccg cctgcgctc caccacgggc caggctcgt gtgcggacta tggcattacg 789

gactgaacc ctctccgc caatgatctg actcccgagc aaaaggctgc cgcggctgcg 849

ctccggcgc cggcggctgc agccatcgtg gcgggtcaa agcagaactg cgagcccgac 909

ctcatgcct acgcccgcc cttgcagta ggcaaaagga cctgctccg catcgtcacc 969

ccc 972

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<213> Candida antarctica

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<221> sig_peptide

<222> (-51)..(-1)

<223> secretion signal

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gatgcgggtc tgacctgcca ggggtcctcg ccatcctcgg tctccaaacc catcctctc	129
gtccccgaa ccggcaccac aggtccacag tcgttcgact cgaactggat cccctctct	189
gcgcagctgg gttacacacc ctgctggatc tcaccccgcc cgttcattgt caacgacacc	249
caggtaaca cggagtacat ggtaacgcc atcaccacgc tctacgctgg ttgggcaac	309
aacaagctc ccgtgctcac ctggtccag ggtggtctgg ttgcacagt ggtctgacc	369
ttctcccca gtatcaggtc caaggatgat cgactatgg ccttgcgcc cgactacaag	429
ggcaccgtc tcgccggccc tctgatgca ctgcgggta gtgcaccctc cgtatggcag	489
caaaccaccg gtcggcact cactaccgca ctccgaaacg cagggtgtct gaccagatc	549
gtgccacca ccaacctcta ctggcgacc gacgagatcg ttacgctca ggtgtccaac	609
tcgcactcg actcatcta cctcttaac ggaaagaacg tcaggcaca ggctgtgtgt	669
gggccgcagt tcgtcatoga ccatgcaggc tcgctcacct cgcagttctc ctacgtctc	729
ggtgatccg ccctgcgctc caccacgggc caggctcgtg gtgcagacta tggcattacg	789
gactgaacc ctctcccg ccaatgatctg actcccgagc aaaaggctgc cgcggctgcg	849

ctcctggcgc cggcggtgc agccatcgtg gcgggtcaa agcagaactg cgagcccgac 909

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ccc 972

<210> 9

<211> 341

<212> PRT

<213> Candida antarctica

<220>

<221> SIGNAL

<222> (-24)..(-8)

<223> secretion signal

<400> 9

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly

-24 -20 -15 -10

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala

-5 1 6

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly

11 16 21

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr

26 31 36

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser

41 46 51 56

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met

61 66 71

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
76 81 86

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
91 96 101

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
106 111 116

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
121 126 131 136

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
141 146 151

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
156 161 166

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
171 176 181

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
186 191 196

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
201 206 211 216

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
221 226 231

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
236 241 246

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
251 256 261

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro

266 271 276

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp

281 286 291 296

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser

301 306 311

Gly Ile Val Thr Pro

316

<210> 10

<211> 341

<212> PRT

<213> Candida antarctica

<220>

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<222> (-24)..(-8)

<223> secretion signal

<400> 10

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly

-24 -20 -15 -10

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala

-5 1 6

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly

11 16 21

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr

26 31 36

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser

41	46	51	56
Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Phe Met			
61	66	71	
Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr			
76	81	86	
Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp			
91	96	101	
Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser			
106	111	116	
Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys			
121	126	131	136
Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro			
141	146	151	
Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg			
156	161	166	
Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser			
171	176	181	
Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp			
186	191	196	
Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys			
201	206	211	216
Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe			
221	226	231	
Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala			
236	241	246	

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
251 256 261

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Pro Ala Pro
266 271 276

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
281 286 291 296

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
301 306 311

Gly Ile Val Thr Pro
316

<210> 11
<211> 341
<212> PRT
<213> Candida antarctica

<220>
<221> SIGNAL
<222> (-24)..(-1)
<223> secretion signal

<400> 11
Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
-24 -20 -15 -10

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
-5 1 6

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
11 16 21

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
26 31 36

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
41 46 51 56

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Phe Met
61 66 71

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
76 81 86

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
91 96 101

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
106 111 116

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
121 126 131 136

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
141 146 151

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
156 161 166

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
171 176 181

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
186 191 196

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
201 206 211 216

Gly Pro Gln Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
221 226 231

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
236 241 246

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
251 256 261

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Ala Leu Leu Ala Pro
266 271 276

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
281 286 291 296

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
301 306 311

Gly Ile Val Thr Pro
316

<210> 12
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<213> Artificial Sequence

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<212> PRT

<213> Artificial Sequence

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<223> a-amylase secretion signal

<400> 13

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Ala Pro Ala Leu Ala

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<210> 14

<211> 317

<212> PRT

<213> Candida antarctica

<400> 14

Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu

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Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys

20 25 30

Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe

35 40 45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys

50 55 60

Trp Ile Ser Pro Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr

65 70 75 80

Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn

85 90 95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln
100 105 110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu
115 120 125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu
130 135 140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly
145 150 155 160

Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile
165 170 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro
180 185 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys
195 200 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Leu Phe Val Ile Asp His
210 215 220

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala
225 230 235 240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr
245 250 255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val
260 265 270

Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ile Val Ala Gly
275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe
290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro
305 310 315

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<220>
<223> LQ53 primer

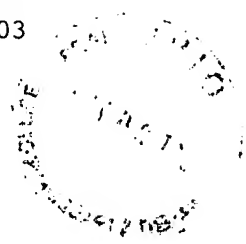
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<210> 17
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<212> DNA



<213> Artificial Sequence

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<223> LP53 primer

<400> 17

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30

<210> 18

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> LP35 primer

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29